STUDIES ON FEEDING, DIGESTION, AND FOOD STORAGE IN FREE-LIVING FLATWORMS (PLATYHELMINTHES: TURBELLARIA)

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Investigations upon turbellarian nutrition (summarized by Hyman, 1951 and Yonge, 1954) have so far dealt mainly with triclads, where intracellular digestion has been demonstrated but the possibility of some supplementary intraluminar digestion not fully explored. Triclad nutrition has therefore been re-examined, using *Polycelis cornuta,* and the opportunity taken to make comparable investigations on representatives of the other turbellarian orders. In each case the nature of the food and the feeding mechanism, the structure of the gut and the course of digestion, and the nature and location of the food reserves have been studied. The various mucoid and rhabdoid secretions have also been examined and an assessment made of their value in feeding.

Materials and Methods

The following Turbellaria, listed systematically with details of their habitat, were examined.

Order Acoela. *Convoluta paradoxa,* Oersted. From rock pools rich in *Ulva* and *Enteromorpha* in Cullercoats Bay, Northumberland.

Order Rhabdocoela. Sub-order Notandropora. *Macrostomum* sp., Oe.

Sub-order Opisthandropora. *Stenostomum* sp., Duges. Both from ponds in the Leeds area.

Sub-order Lecithophora. *Mesostoma tetragonum,* O. F. Müller. From Middlerigg Tarn, Troutbeck, Westmorland.

Order Tricladida. Sub-order Paludicola. *Polycelis cornuta,* Schmanda. From under stones in small fast-running streams in the Leeds area.

Order Polycladida. Sub-order Cotylea. *Cycloporus papillosus,* Lang. On colonial tunicates (*Botryllus* and *Botrylloides*) beneath weed-covered rocks at low-water mark on St. Mary's Island, Northumberland.

Sub-order Acotylea. *Leptoplana tremellaris,* O. F. M. Beneath stones on a predominantly sandy shore at Llanfairfechan, North Wales.

The flatworms were starved to encourage a readiness to feed and to clear the gut lumen and the gut cells of any remnants of previous meals. The animals associated with the flatworms under natural conditions were then presented to them so that the methods of capture and ingestion of the selected prey could be followed in detail. Flatworms were killed where possible in the act of feeding, Steinmann's fixative (equal parts concentrated nitric acid, distilled water and saturated mercuric chloride in 5% aqueous sodium chloride) giving the necessary instantaneous fixa-

tion, and after washing in running water the preparations were stained in borax carmine.

The natural food was used, whenever possible, to determine the site and course of digestion, but in forms with a phagocytic gastrodermis (especially the triclad) both this and the soft foods used by previous investigators (Arnold, 1909; Willier, Hyman and Rifenburgh, 1925; Kelley, 1931) disintegrated during ingestion and it could not be ascertained whether any intraluminar break-up preceded the phagocytosis and intracellular digestion, nor whether the separate constituents of the food (proteins, carbohydrates and fats) were dealt with differentially. Hence the flatworms were fed either on food which reached the gut in a visibly recognizable condition, or on homogeneous food substances whose digestion could be detected by simple chemical tests. Some of these were readily eaten, but with others the natural prey had to be used as a carrier. This particularly useful technique was applied by extracting the body contents of a normal prey with a hypodermic syringe and replacing them with the appropriate food substance. In all cases the flatworms were fixed at intervals after an observed feed, and examined histologically. All fixation (in Susa, saturated mercuric chloride or 10% formalin) was carried out at about 40° C. to prevent rupture or discharge of the gut contents. Sections cut at 10 μ were stained with Ehrlich's haematoxylin and eosin, Heidenhain's iron haematoxylin and Feulgen's stain. Squash preparations were also made, either directly in 0.5% saline or after preliminary maceration in saturated aqueous boric acid, and examined both fresh, and fixed and stained. In cases where ingested food was visible within the living flatworm its fate was also followed by direct observation.

Fat reserves were studied from squashes fixed in osmium tetroxide vapour and from sections prepared after fixation in Flemming's fluid. Carbohydrate reserves were studied after fixation in 95% alcohol, the sections being stained with Best's carmine for glycogen or periodic acid-Schiff's reagent for carbohydrates generally. For protein reserves flatworms were fixed in neutral 10% formalin and the sections stained by the modified Millon method (Bensley and Gersh, 1933).

Special methods for the study of pH changes during digestion and the examination of the mucoid and rhabdoid secretions are described in the text.

Observations

TRICLADIDA. *Polycelis cornuta*

The food and feeding mechanisms

P. cornuta (1–1.5 cm. long and 2–3 mm. broad) feeds upon small annelids, crustaceans and insect larvae, particularly those of Ephemeroptera, Plecoptera and Diptera. It is also a scavenger and feeds upon injured or dead animals, provided they are not too decomposed—juices diffusing from such animals attract all the flatworms in the vicinity and they can be collected in great numbers by anchoring a crushed earthworm in the stream as bait.

The living prey may be seized directly but is often caught after it has become entangled in the mucus produced for locomotion and adhesion by the *Polycelis* population in general. The normal slow movement by ciliary gliding is facilitated by mucus secretions which are laid down in tracts comparable to the slime trail of a snail; this mucus is not particularly sticky but small animals are occasionally trapped

in it. Often, however, ciliary locomotion is supplemented by direct muscular movement in which waves of contraction pass down the body and urge it forward. During such movement, and particularly when the flatworm is facing a current or changing levels upon a plant or stone, mucus of the type usually used for adhesion when at rest is produced from the edges of the body to provide temporary anchorage during the muscular contractions. This mucus is very sticky and as the flatworm moves it is drawn out into strands 2–3 cm. long which stretch between stones and leaves or along a level substratum. The many single strands produced in this way tend to cross or coalesce to form a complex tangle which becomes a most effective trap for small animals. Such deposits occur all over the natural habitat, and can be easily demonstrated in laboratory culture dishes by flooding with weak eosin (Fig. 11). *Polycelis* is gregarious and this habit results in a large amount of mucus being deposited within a given area. Animals with many appendages, such as crustaceans and insect larvae, are easily trapped in these mucus "snares" and become still more entangled in their struggle to escape. The flatworm does not lie in wait near the "snares" but is rapidly attracted by any disturbance in the water created by the struggles of the prey. Several individuals are often attracted to the same prey and their concerted efforts enable them to feed upon animals too large for a single individual. The prey is seized by the flatworm wrapping itself about it and covering it with large amounts of the sticky mucus. In this way even relatively large animals such as gammarids are rapidly immobilized. There is no evidence of any toxic effects of the mucus since prey rescued after complete immobilization show no ill effects. Inert food does not stimulate the formation of much mucus; only the amount necessary for adhesion is secreted when such food is being eaten. After the prey is seized the pharynx is protruded and makes exploratory movements over the body surface. With arthropods it is eventually forced through some weak point in the exoskeleton, as between sclerites or the area of articulation of a limb, and rapidly sucks out the body contents (Fig. 10). The pharynx is very extensible and moves about within the prey, entering the head and larger appendages to draw out all the organs and musculature, so that virtually only the empty exoskeleton is finally left. With annelids the cuticle is breached and the body of the worm extracted, whilst with suitable carrion minute pieces are sucked off by the pharynx. The disintegration of the food on its way to the gut is so rapid that it appears to be due entirely to mechanical disruption during its passage through the pharynx; there is no evidence that digestive juices assist the process since the waves of muscular contraction in the pharynx invariably pass backwards towards the gut, never forwards as would be expected if such juices were being regurgitated. The efficacy of the process is indicated by the homogenized condition of the food as it arrives in the gut lumen.

The structure of the gut and course of digestion

The large cylindrical plicate pharynx lies in the pharyngeal chamber in the posterior half of the body and is directed backwards so that it can be protruded through the posterior central "mouth" by simple muscular elongation (Fig. 1). It is very muscular and contains both eosinophilic and basophilic mucus glands whose secretions help ingestion.

The gastrodermis (Figs. 12 and 14) consists of a single layer of cells resting upon a delicate basement membrane. There are two types of cells, of which the

larger and more numerous are columnar, 35–40 μ in height with basal vesicular nuclei; their distal ends often bulge freely into the gut lumen and their basophilic cytoplasm is finely granular and may contain various eosinophilic inclusions. These inclusions disappear progressively after feeding and hence appear to represent phagocytosed food undergoing intracellular digestion. The second type of gut cell, later referred to as "sphere cells" on account of their appearance, is 20–30 μ high and normally contains intensely staining spheres which appear to constitute a protein reserve.

The finely divided condition and mixed nature of the normal food after ingestion made it difficult to determine whether phagocytosis was preceded by any intraluminar break-up, and experimental feeding by the carrier technique was applied.

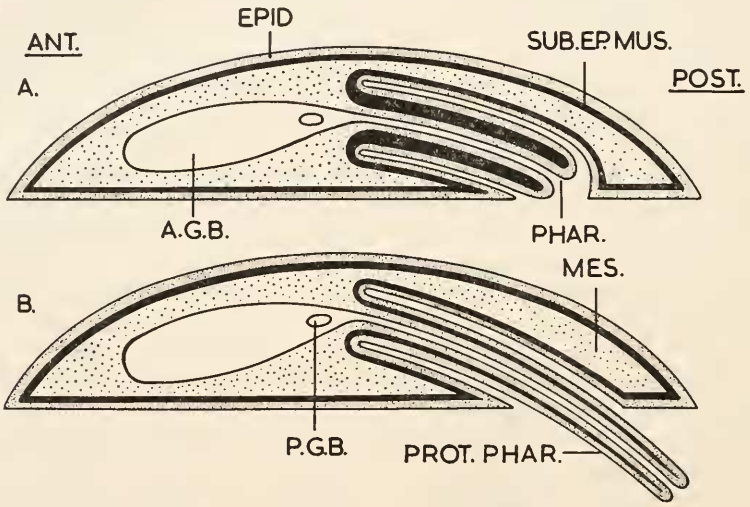


FIGURE 1. Diagrammatic longitudinal sections of *Polycelis* to show the cylindrical plicate pharynx. A, The normal condition with pharynx retracted. B, Pharynx protruded for feeding; ant.: anterior; a.g.b.: anterior gut branch; ev.phar.: everted pharynx; m.g.b.: median gut branch; mes.: mesenchyme; phar.: pharynx; post.: posterior; p.g.b.: origin of posterior gut branches; prot.phar.: protruded pharynx; sub.ep.mus.: subepidermal muscles.

The carriers used in this case were gammarids which were boiled before injection to ensure that no active enzymatic juices of the crustacean remained to attack the test food. The series of test foods was chosen with three objectives: (a) to follow the separate fates of carbohydrates, proteins and fats; (b) to supply unequivocal evidence of the presence or absence of intraluminar digestion; and (c) to determine the maximum size of particle which can be phagocytosed by the gut cells.

(1) Experimental feeding with carbohydrates

Polycelis readily ingested boiled starch paste, and sections prepared immediately after feeding and stained with Lugol's iodine showed the blue and unchanged starch lying in the gut lumen. The paste was quickly phagocytosed and twelve hours after feeding only a small amount remained in the lumen, quite unaltered and still stained blue with Lugol, indicating the absence of any intraluminar digestion of carbohy-

drates. The intracellular digestion of the phagocytosed starch was followed in saline squashes of fed flatworms. Two hours after feeding the columnar gut cells contained large amounts (Fig. 13), which stained with Lugol in various shades of blue and brown. As time passed all stained brown and then eventually disappeared as digestion was completed. Starch was also found in mesenchymal cells so that a certain amount of phagocytosed material passes back into the mesenchyme for digestion. Indicators mixed with the starch paste were unchanged in the gut lumen, giving further evidence of the absence of intraluminar digestion, but as the pH value after phagocytosis was 6.8–7, intracellular digestion of carbohydrates occurs in a neutral to slightly acid medium.

Raw starch grains were ingested and readily phagocytosed if sufficiently small, and could be seen very clearly in sections by polarized light in the columnar cells. They were unaffected by the intracellular diastatic enzymes and were eventually rejected by the cells and returned to the gut lumen. In this case, therefore, no capacity for the selection of suitable food material appears to be possessed by the gut cells themselves. On the other hand, no grains above about 30 μ were taken up and apparently this is the critical size above which phagocytosis is impossible. Later all the starch grains were expelled in the usual manner by taking in water through the pharynx and flushing out the gut contents by violent contractions of the body.

(2) Experimental feeding with proteins

Polycelis fed directly upon clotted frog and chick blood. The erythrocytes were quickly phagocytosed and could be seen in the columnar gut cells in saline squashes made thirty minutes after feeding. The number phagocytosed increased with time and six hours later almost every cell was packed full. Occasionally erythrocytes were also seen in mesenchymal cells. The erythrocytes quickly changed in appearance after phagocytosis and condensed into homogeneous deeply staining spheres which decreased in size and number as digestion and absorption progressed (Fig. 14). They showed no signs of digestion in the gut lumen prior to phagocytosis.

Chopped frog muscle stained with various indicators was fed to the flatworms by means of gammarid carriers. The particles reached the gut in recognizable pieces still showing their characteristic striations and the larger remained unchanged in size, shape and pH value until ejected from the gut some 24 hours later. This indication of the absence of intraluminar proteolysis was confirmed by feeding coagulated albumen, gelatin and fibrinogen, all of which likewise remained unchanged throughout their stay in the gut lumen. The smaller particles of chopped muscle were phagocytosed and neutral saline squashes made one hour after feeding showed many present in the columnar gut cells. Particles stained with brom-cresol green were then a clear blue in color (pH 5.2) but changed with time until six hours after feeding when they had become a clear green (pH 4.6), so that intracellular protein digestion proceeds in a distinctly acid medium. This color faded as the particles themselves became rounded and less distinct. Stained inclusions passing through the same series of color changes were occasionally found in mesenchymal cells. This indicator incidentally proved to be most attractive to these flatworms and they ingested large amounts of food stained by it; particles of the indicator dropped into culture dishes attracted the flatworms to them and stimulated protrusion of the pharynx.

(3) Experimental feeding with fat

Large amounts of cod liver oil suspensions stained with Sudan IV were readily ingested from carrier gammarids, often increasing the body volume of the flatworms by 40–50%. Saline squashes and frozen sections prepared thirty minutes after feeding showed some stained oil globules within the columnar cells, and the number increased with time. Irrigation of the preparations with Nile Blue (Smith, 1907; George, 1951) showed the globules changing from red to deep blue as the stain penetrated the cells showing that much of the phagocytosed oil was undergoing intracellular lipolysis with the production of free fatty acids. Nothing suggesting intraluminar lipolysis was seen. Stained globules appeared early in the mesenchymal cells, and later became much more numerous, but only a few ever showed any color reaction with the Nile Blue. Since the Sudan IV stains the fatty acid radical of fat molecules and remains with it through lipolysis and resynthesis, it appears probable that the red globules in the mesenchyme cells had mainly been broken down and re-formed in the gut cells into the flatworm's own particular type of fat and then passed into the mesenchymal cells for storage. Flatworms with this stored fat had a diffuse pink color which persisted for several weeks and faded only gradually as the fat was metabolized and the Sudan IV excreted. Saline squashes made six weeks after feeding still showed occasional red globules in the mesenchymal and columnar gut cells. It was not possible to determine the pH conditions attending intracellular lipolysis since indicators dissolved in the water of the cod liver oil suspension were not taken up by the cells. Suet particles were also fed. The largest particles remained in the gut unchanged in size and shape until ejected 24 hours later, confirming the absence of intraluminar lipolysis. The smaller particles were phagocytosed and digested intracellularly.

The nature and location of the food reserves

Polycelis forms large reserves of protein and fat, supplemented by some carbohydrate reserve, and these enable the flatworm to survive for at least three months without food.

The gonads, mesenchyme and general body tissues constitute a protein reserve which is drawn upon during starvation, with a consequent reduction in body size, but there are also specific protein reserves in the sphere cells of the gastrodermis. These contain 8–16 small homogeneous concentrations which normally stain deeply with modified Millon, eosin and iron haematoxylin, but their appearance and staining affinity varies with the nutritive state of the animal. In well-fed laboratory individuals, and in those collected out of doors in late summer and well nourished in preparation for winter, the spheres are all compact and densely staining. During starvation they become increasingly indistinct, stain poorly and finally disappear. At the same time a progressive reduction in the volume of both columnar and sphere cells results after about three months in a flattened gastrodermis devoid of any inclusions. The significance of the sphere cells as protein reserve can be demonstrated by comparing the proportion of sphere and columnar cells under a variety of nutritive conditions. These are summarized in Table I which is compiled from cell counts of every third section of a number of histological series.

The fat reserve is laid down as globules in the inner mesenchyme and columnar

gut cells. It decreases only slowly during starvation, and significant amounts still remain after three months without food.

The carbohydrate reserve occurs as small irregular granules of glycogen, scattered throughout the mesenchyme and columnar cells. It decreases rapidly on starvation and disappears from the mesenchyme after about a fortnight. Small amounts persist in the gut cells, however, for much longer than this, but these may possibly result from the conversion of other reserves. A purely carbohydrate diet results in an enormous increase in the amount of glycogen in the mesenchyme and the flatworms, provided they were mature, survived as well on this diet as upon purely protein or fat diets.

The epidermal and subepidermal glands and their relation to feeding

These secretions can be divided into mucoid, giving a positive reaction with P.A.S. and Alcian Blue (Steedman, 1950), and the non-mucoid or rhabdoid, giving a negative reaction.

The mucoid can be further differentiated into eosinophilic and basophilic. The latter is produced by scattered gland cells in the epidermis and outer mesenchyme,

TABLE I

The proportion of cell types in relation to nutrition

Nutritive condition	Ratio of "sphere" to columnar cells
High protein diet	930:3723 = 1:4
Normal diet	625:3957 = 1:6.3
Starvation: 14 days	435:2957 = 1:6.7
Starvation: 28 days	371:3545 = 1:9.5
Starvation: 2 months	176:2668 = 1:15.5
Starvation: 3 months	"Sphere cells" absent and gastrodermis syncytial

and is used in gliding and to a minor extent in trapping prey as already mentioned. There are no large aggregations of basophilic glands as described in some other triclads. The much more adhesive eosinophilic mucus is produced both from scattered gland cells and from concentrations of mesenchymal gland cells, the "marginal adhesive glands" of Hyman (1951), situated along the borders of the ventral surface. During its discharge the epidermis becomes elevated into small temporary papillae so that the secretion is laid down in lines of oval deposits, from which are formed the main elements of the "snares."

The non-mucoid rhabdoids are transparent greenish rods, 15–20 μ in length, produced by mesenchyme cells and passed out to the epidermis. Once outside the body the rhabdoids take up many times their own volume of water ("hydrate") and fuse to form a semi-fluid gelatinous layer which closely invests the body. This material can be collected for examination in two ways. It can be removed from the living flatworm by drawing a fine glass needle a number of times across the dorsal surface of the body. Alternatively, immersion of the worm in strong sodium chloride solution causes the discharge of enormous numbers of rhabdoids and at the same time inhibits their hydrolysis, so that they can be fixed in 95% alcohol for histological examination. Irrigation of unfixed rhabdoids with water causes them to hydrate rapidly (Figs. 15 and 16), producing the same sort of gelatinous sheet as invests

the normal flatworm. The lowest critical concentration of salt to give discharge without hydration is about 3.75% ; possibly hydration within the epidermal cells may be inhibited by some comparable combined effect of the cytoplasmic salts. The rhabdoid material is basic (pH 8.0–8.2), soluble in acids and alkalies, and stains strongly with Millon; apparently it is of a purely protein nature, as all fat and carbohydrate tests were negative. The hydrated material is not toxic to other animals, annelids and crustaceans surviving for long periods in contact with it, and it plays no part in feeding. It is distasteful to fish, pieces of earthworm smeared in it being rejected by sticklebacks, but its main function is probably mechanical, acting as a "fluid cuticle" to protect the epidermis from abrasion and bacterial and fungal attack whilst still permitting ciliary activity, and in life it is probably in a continual state of loss and renewal. There is also a rapid discharge of rhabdoids if a flatworm is injured, to give a protective clot-like effect over the region of the wound.

ACOELA

Convoluta paradoxa (2–3 mm. long and pear-shaped with a pronounced ventral curling of the broader anterior end) feeds upon small marine crustaceans, protozoa, diatoms and similar organisms which are captured by various methods, depending upon their size. Minute prey such as protozoa are captured by the flatworm gliding slowly over algal fronds or stones with the solid syncytial gut partially extruded through the mouth like a large pseudopodium and the food engulfed amoeboid fashion and passed back into the syncytium for digestion. Alternatively the flatworm may spend long resting periods attached to the substratum by the adhesive papillae of the tail and with the rest of the body slightly raised in a "sitting-up" position. If larger prey, such as crustaceans or their larvae, come sufficiently close, the flatworm rapidly extends its body and grasps the prey with the curved anterior margin, which is covered with abundant sticky mucus produced by the frontal and other subepidermal glands. The flatworm then curls up ventrally, starting from the anterior end, so that the prey is drawn beneath it and pressed into the mid-ventral mouth. This is very distensible and the food is ingested whole and intact. More rarely *Convoluta* captures actively swimming creatures whilst it is itself either swimming freely or gliding over the algal fronds, but in all cases capture and ingestion are extremely rapid and occupy only 5–10 seconds. The flatworm shows marked avoiding reactions when dead food is encountered; it feeds only upon living organisms and does not act as a scavenger.

The simple pharynx is a very short ciliated tube leading directly to the solid "gut"—a central compact or vacuolated syncytium, one-third to one-half of which can be protruded through the mouth (Fig. 5). Its cytoplasm is finely granular and contains many vesicular nuclei. All prey when ingested is enclosed in temporary vacuoles which follow no definite path, but merely move back inside the animal to come to rest in any part of the syncytium. Occasionally vacuoles containing digesting prey may be found in the mesenchyme, but the cytoplasm about these vacuoles is always distinct from the surrounding tissue and is apparently a temporarily isolated part of the digestive syncytium. As digestion progresses the food becomes disorganized and breaks up and in Crustacea only fragments of the exoskeleton remain when digestion and absorption are complete, some 18–24 hours after feeding (Fig. 6). These indigestible residues then pass to the mouth to be thrown out.

It was not possible to determine the pH conditions controlling digestion as *Convoluta* refused crustaceans stained with indicator. Attempts to recover food in various stages of digestion for pH examination also failed, as it inevitably became contaminated with mesenchyme, mucus and sea water.

Fat forms the principal food reserve in *Convoluta*, occurring in large amounts as globules of 2–3 μ diameter in the outer, more compact mesenchyme and in the digestive syncytium. Small amounts of glycogen also occur as irregular granules scattered throughout the mesenchyme and digestive tissue. There are no specific protein reserves.

RHABDOCOELA

(1) *Macrostomum* sp. (2–3 mm. long and 0.5 mm. broad) feeds upon minute fresh water crustaceans, annelids, nematodes, rotifers and large ciliate protozoans. The mouth and pharynx are capable of great distension and any small creature moving near the flatworm is seized and ingested whole. Only living food is taken and mucus plays no part in its capture.

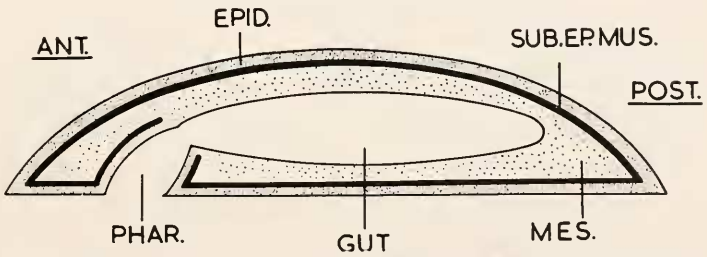


FIGURE 2. Diagrammatic longitudinal section of *Macrostomum* to show the simple pharynx. Abbreviations as in Figure 1.

The ventral slit-like mouth opens into a simple ciliated pharynx longer than that of *Convoluta* but still with no thickening of the underlying muscular layers (Fig. 2). Eosinophilic glands in the mesenchyme open into the pharynx but they are not mucus-producing and their precise function remains unknown. The gut is a simple unbranched sac extending almost the whole length of the body and its wall is made up of two kinds of cells, standing upon a thin basement membrane (Fig. 7). The larger and more numerous are columnar, some 30–40 μ tall and 5–6 μ broad, with basal vesicular nuclei and finely granular cytoplasm which may have inclusions, giving the cell a phagocytic appearance. The second type of cell is smaller and bears a close resemblance to the "sphere-cell" of the triclad gastrodermis. It is club-shaped, 15–20 μ tall and 3–4 μ broad distally, with the nucleus in the narrower basal region where the cytoplasm is often strongly basophilic. These cells contain 10–15 eosinophilic spheres which also stain with iron haematoxylin and modified Millon.

Sections of *Macrostomum* fixed 15 minutes after feeding on *Daphnia* showed the intact crustaceans lying free in the gut. In others fixed 10 hours later digestion was well advanced, with the muscles and organs greatly disorganized, whilst 24 hours after feeding only the exoskeleton remained, but as the latter retained its general shape there can be little movement or contraction of the gut during digestion.

This was confirmed by observation of living flatworms. During digestion the columnar gut cells became shortened, swollen and indistinct from one another, probably as a result of secretory activity or absorption of digested food, or both. The "sphere cells" remained unchanged. These observations confirmed the presence of intraluminar digestion in *Macrostomum* but after feeding upon *Paramecium* or small annelids almost all the gut cells showed discrete inclusions which were clearly particles phagocytosed from the disintegrating food mass in the gut lumen (Fig. 7). Thus digestion in *Macrostomum* must be a combination of intraluminar and intracellular processes, the latter occurring when the food in the lumen is sufficiently disintegrated for the particles to be phagocytosed. The small amount of phagocytosis and intracellular digestion seen with crustaceans may be due to the exoskeleton retaining most of the disintegrating material until it is rendered completely soluble. The pH conditions of digestion could not be determined as the flatworms refused to ingest prey stained with indicator.

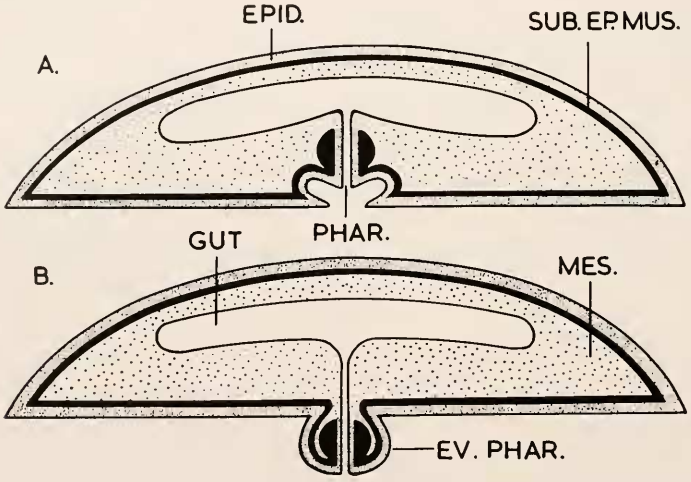


FIGURE 3. Diagrammatic longitudinal sections of *Mesostoma* to show the bulbous pharynx. A, The normal condition with the pharynx retracted. B, Pharynx everted for feeding. Abbreviations as in Figure 1.

Small amounts of glycogen and fat occur in the columnar gut cells and in the mesenchyme, whilst the "sphere-cells" of the gut epithelium appear to constitute a definite protein reserve, since they disappear after 10–14 days' starvation.

(2) *Stenostomum* sp. (1.5–3 mm. long and very slender) feeds mainly upon large ciliates and rotifers, which are seized in the anterior ventral mouth and engulfed whole. These animals are usually found by chance but the flatworm may search actively for them, crawling slowly forwards with the anterior end slightly raised and the mouth wide open. The latter is capable of great distension and is fringed with large cilia which probably help to sweep the prey back into the pharynx, where it is retained a few seconds for examination. If unsuitable it is rejected, but if acceptable the mouth is closed and the pharynx contracts violently to force the food into the gut. The pharynx is a simple ciliated tube, similar to that of *Macrostomum*, with some basophilic gland cells producing mucus to help ingestion. The

gut is a simple blind sac extending almost to the posterior end of the body, its anterior end is contracted and open communication with the pharynx exists only during actual ingestion. The gastrodermis is of unciliated club-shaped cells, 20–25 μ tall and 6–8 μ broad distally with basal nuclei and granular inclusions.

When ingestion is complete, and the pharynx again closed off, the gut contracts violently and the food is driven to and fro in the lumen (Fig. 9). Ciliates are broken up within fifteen minutes, and indeed are often disintegrated by the pharynx during ingestion. Rotifers resist longer but eventually their body contents are squeezed out through the mouth or some weak spot in the integument and the empty cuticle ejected. The resultant particles are phagocytosed by the gut cells and show for a time as eosinophilic inclusions until intracellular digestion is completed. Occasionally *Paramecium* stained with indicators covering a pH range of 3.5–8.4 were accepted but during their disintegration the pH remained unaltered, showing the absence of intraluminar digestive activity.

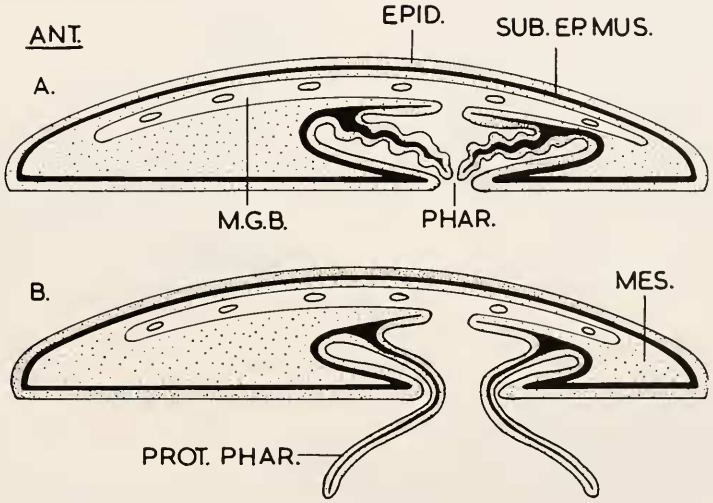
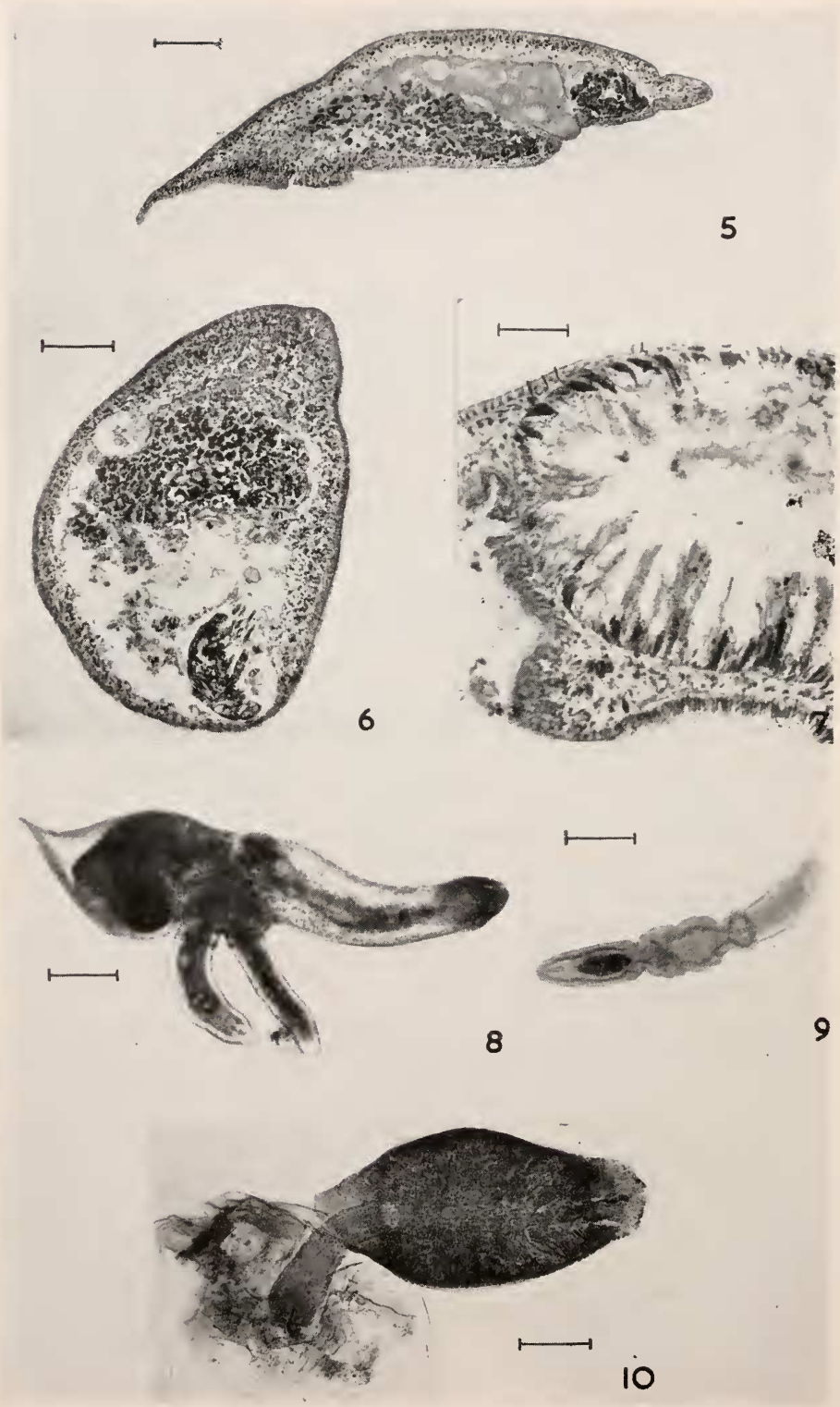


FIGURE 4. Diagrammatic longitudinal section of *Leptoplana* to show the ruffled plicate pharynx. A, The normal condition with pharynx retracted. B, The pharynx protruded to envelop food. Abbreviations as in Figure 1.

Fat forms the only food reserve in *Stenostomum*, occurring as small globules in the gut cells. The amount decreases rapidly and little remains after a week, which is about the maximum period the flatworm can endure without food.

(3) *Mesostoma tetragonum* (5–8 mm. long and 1–2 mm. broad) feeds upon small oligochaetes, crustaceans and insect larvae, which are captured directly by the flatworm wrapping itself around them. The bulbous pharynx is then protruded through the median ventral mouth (Fig. 3) and applied to the prey's body wall. Strong sucking movements made by alternate contractions and expansions of the radial musculature of the pharynx draw small prey bodily into the gut (Fig. 8). Larger animals are held until the body wall ruptures and the pharynx can be thrust into the body to suck out the contents, but in a somewhat inefficient manner so that frequently only the body fluids are withdrawn. *Mesostoma* is also a



FIGURES 5–10.

scavenger and will feed upon any sort of dead prey, if not excessively decomposed. The gut is a simple sac and the gastrodermis is syncytial, 15–20 μ thick, often with spherical inclusions.

The ingestion of whole prey implied that digestion in *Mesostoma* is at least partially intraluminar and this was confirmed by experimental feeding with clotted frog blood. The erythrocytes are largely broken up within thirty minutes, though their freed nuclei are still distinct, but there is no evidence of phagocytosis by the gut cells during this initial period. After three hours the material in the gut lumen has become a homogeneous mass, though a light reaction with Feulgen indicates that the ruptured nuclei are not yet entirely decomposed, and as this stage is reached material of similar appearance begins to show as small spheres within the gut cells. After six hours the lumen was empty and the gut cells packed with spheres, which in turn decreased and disappeared within 24 hours. Intracellular digestion in *Mesostoma* is therefore preceded by some degree of intraluminar digestion, but details are unknown as no successful method of feeding with indicators could be found.

POLYCLADIDA

(1) *Cycloporus papillosus* (1–2 cm. long and broadly oval) is usually found attached by its single ventral sucker to the surface of the encrusting colonial tunicates *Botryllus* and *Botrylloides*, and it appears to feed exclusively upon these animals. The anterior cylindrical plicate pharynx, shorter but otherwise resembling that of *Polycelis*, is protruded forwards and thrust down into the colony to suck out individual zooids. The pharynx leads into the median gut branch which gives off 6–8 pairs of lateral diverticula, subdividing in turn to give the typical polyclad arrangement. The distal gut branches lead up to large "anal chambers," ranged along the margins and opening by pores through the epidermis. In histological preparations the branches and anal chambers always appear closed off from each other, and no actual passage outwards of gut material has ever been found; possibly temporary openings may develop for the passage of excess water taken in with the food.

The majority of the gastrodermal cells are columnar, 35–40 μ tall and 5–8 μ wide, with their free margins ciliated, the cilia being particularly strong in the median diverticulum (Fig. 17). There is, however, an anomalous region of columnar cells at the posterior end of this diverticulum where the cilia are replaced by what appears to be a "brush border." Scattered amongst the columnar cells are smaller

FIGURE 5. Longitudinal section of *Convoluta* showing the central vacuolated syncytium. Haematoxylin and eosin. Scale 0.25 mm.

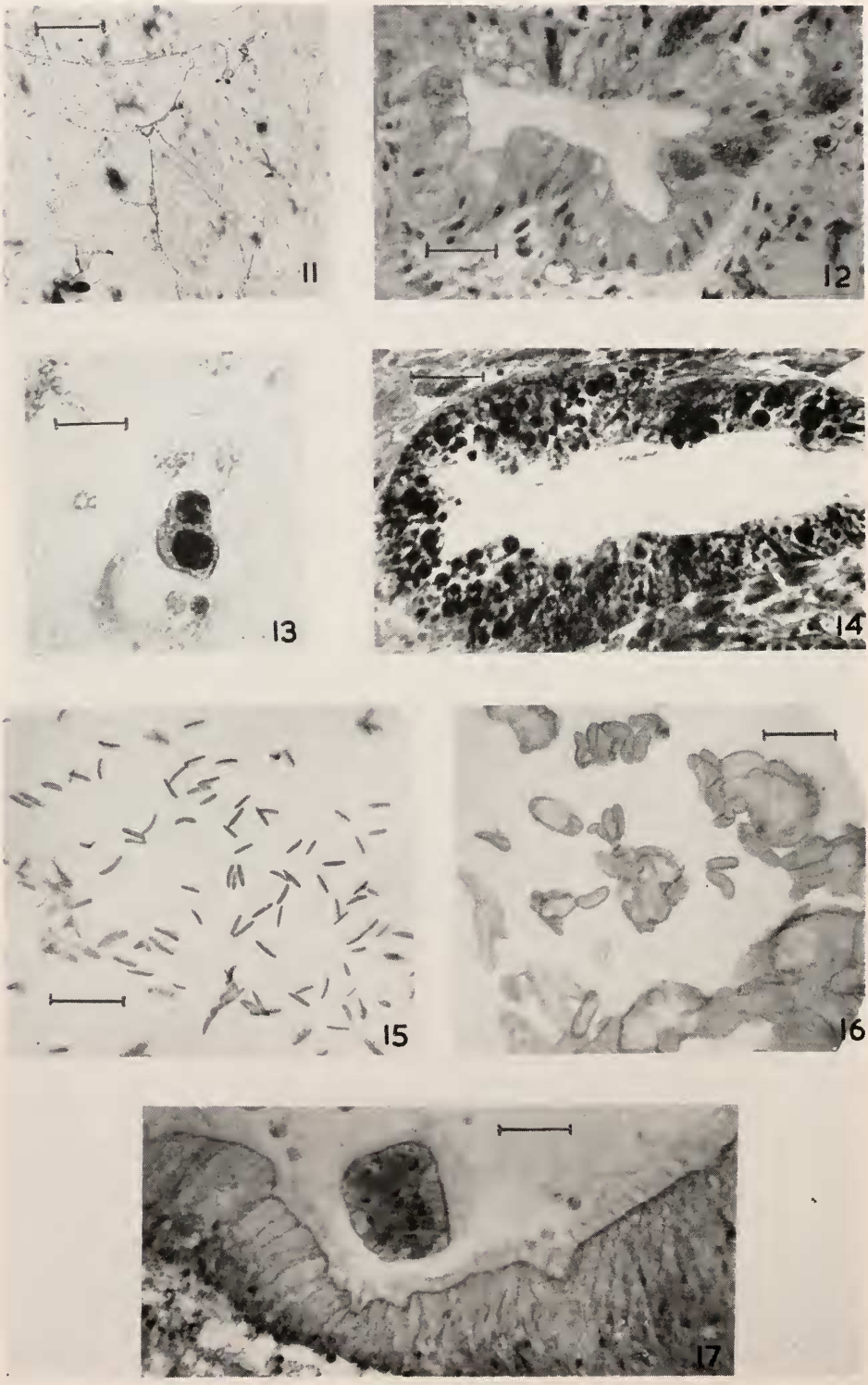
FIGURE 6. Transverse section of *Convoluta* showing a recently ingested intact crustacean and one completely digested except for the exoskeleton. Haematoxylin and eosin. Scale 0.1 mm.

FIGURE 7. Longitudinal section of *Macrostomum* showing part of the simple pharynx (left) and the gastrodermis with small, club-shaped "sphere cells" between the long, projecting phagocytic cells. Haematoxylin and eosin. Scale 20 μ.

FIGURE 8. *Mesostoma* ingesting an annelid. From life. Scale 1 mm.

FIGURE 9. *Stenostomum* immediately after ingesting a *Paramecium* stained with neutral red. The gut is beginning to contract to break up the ciliate. From life. Scale 0.4 mm.

FIGURE 10. *Polycelis* killed whilst feeding on a large *Daphnia*. Note the protruded pharynx. Steinmann's fixative and borax carmine. Scale 1 mm.



FIGURES 11–17.

numbers of spherical glandular cells, up to 45 μ in diameter, their cytoplasm laden with tiny granules. These cells occur in various sizes, the smaller appearing between the bases of the columnar cells and the larger nearer the lumen; occasionally they were seen free in the lumen, and their disintegrated remains were found both here and between the bases of the columnar cells. Their phases of growth and disintegration, however, could not be related in any way to the condition or amount of food in the gut and it is not at all certain that they are concerned in digestion. The columnar cells themselves, on the other hand, do appear to secrete juices into the lumen, becoming swollen and vacuolated soon after feeding.

In feeding, the tunicate zooids reach the gut almost undamaged; histological examination at intervals after an observed feed shows a progressive homogenization of the food, leading to complete absorption after about twelve hours. At no time is there any indication of other than intraluminar digestion. Confirmation came from feeding starved *Cycloporus* on *Botryllus* colonies injected with starch paste; Lugol sections showed digestion confined within the lumen, with unchanged starch never appearing within the gut cells. Food containing indicators, however, was consistently refused. Fat globules in the ciliated cells and mesenchyme constitute the only significant food reserve. Only minute amounts of glycogen occur in the mesenchyme and there are no specific protein reserves. The flatworm cannot withstand more than about seven days' starvation.

Only basophilic mucus glands are present, scattered throughout the epidermis and ventral mesenchyme, and mucus plays no part in feeding. Rhabdoid-producing cells are common in the mesenchyme, particularly between the posterior gut diverticula, and their cycle of activity can be followed in its entirety. Immature cells contain small eosinophilic granules and rods which enlarge into the fully formed rhabdoids. As these are discharged the cells shrink to a fraction of their former size and migrate into the nearest gut diverticulum; there they disintegrate and are presumably expelled with the unwanted food material. The discharged rhabdoids themselves follow one of two courses. Some pass to the epidermal cells and so to the exterior in the usual way. The experimental removal and examination of these rhabdoids gave results identical to those reported for *Polycelis*. Others aggregate to form conspicuous tracts, greyish white in the living flatworm, which converge upon the gonopore. The rhabdoids pass through or between the cells of the gonopore wall and are discharged with the eggs. Sections of the egg masses show the individual eggs to be embedded in a gelatinous matrix which has properties similar to those of hydrated rhabdoids. The rhabdoids are not concerned in any

FIGURE 11. Mucus "snares" produced by *Polycelis*. Alcian blue. Scale 0.5 mm .

FIGURE 12. *Polycelis*. Transverse section of gut diverticulum showing columnar phagocytic cells and smaller heavily staining "sphere cells." Iron haematoxylin. Scale 40 μ.

FIGURE 13. *Polycelis*. An isolated columnar cell with phagocytosed starch. From a saline squash. Lugol. Scale 40 μ.

FIGURE 14. *Polycelis*. Transverse section of the gut diverticulum 24 hours after feeding on chick blood, showing phagocytosed erythrocytes undergoing intracellular digestion. Iron haematoxylin. Scale 40 μ.

FIGURE 15. *Polycelis*. Rhabdoids discharged after immersion in 5% aqueous sodium chloride. Scale 50 μ.

FIGURE 16. *Polycelis*. Rhabdoids discharged as in Figure 15, then irrigated with tap water. Scale 50 μ.

FIGURE 17. *Cycloporus*. Longitudinal section of the median gut branch showing ciliated gastrodermis. Haematoxylin and eosin. Scale 40 μ.

way in the formation of the egg membranes since these stain strongly with P.A.S. and are already fully formed in the oviduct well above the point of entry of the rhabdoids.

(2) *Leptoplana tremellaris* (2–2.5 cm. long and pear-shaped) feeds upon polychaetes, isopods and amphipods which are captured directly without any trapping devices. The prey is seized by the anterior part of the flatworm's body and wrapped round by the expanded body margins. The flatworm then curls up and passes the prey back along the ventral surface to the median mouth. The pharynx is of the ruffled plicate type (Fig. 4), consisting of a large thin convoluted oval curtain suspended from the roof of the peripharyngeal chamber, and is protruded through the mouth to envelop the prey. Small animals are ingested whole, either by simple peristalsis or by the pharynx being drawn back within the mouth, a process requiring five minutes. Larger organisms are enveloped as far as possible and the pharynx is retracted slightly so that the prey is partially ingested; the flatworm then presses itself down on the substratum and the pharynx makes strong sucking movements which are often supplemented by movements of the whole body. This process may continue for an hour or more, whilst the prey is disintegrated and ingested piece by piece or has its integument ruptured and the contents squeezed out. *Leptoplana* also feeds upon dead animal material provided it is not too decomposed.

The gut is of the usual polyclad form but the gastrodermis here consists of unciliated vacuolar columnar cells. In well-fed individuals these are indistinct in outline or even syncytial and the cytoplasm loaded with eosinophilic spheres which disappear on starvation. This appearance of phagocytic activity was confirmed by experimental feeding with frog blood and small pieces of *Littorina* muscle. The former were rapidly taken up by the gut cells and their intracellular digestion and disappearance easily followed. The small pieces of muscle entered the gut entire but subsequent changes in their histological appearance showed that they were partially broken down by enzymatic activity in the gut lumen before entering the gut cells, where their fate follows that of the usual eosinophilic inclusions. In this case, therefore, intraluminar digestion is incomplete and is succeeded by phagocytosis and intracellular digestion.

The possibility that enzymes from the gut could be regurgitated to assist disintegration in the protruded pharynx was investigated by using pieces of *Littorina* foot muscle too large to be ingested whole. These pieces were removed from the flatworm's pharynx after a series of time intervals and checked against control muscle for pH and enzymatic changes. Control muscle was slightly acid (pH 6.6–6.8), but after ten minutes decreased to 5.4 and after twenty to 4.5. For proteolytic activity the muscle was laid on gelatin-coated slides and half an hour allowed for any enzyme to diffuse out. The gelatin below and around a piece of muscle which had been in the pharynx for a quarter of an hour was dissolved away and the holes formed in the gelatin film showed clearly after staining the slide with eosin. Control muscle had no such effect. Corresponding acidity and enzyme production in the gut lumen can therefore be reasonably inferred.

Fat again forms the only significant food reserve, and what has been said of *Cycloporus* about mucus and rhabdoids applies also to *Leptoplana*.

Discussion

The general pattern of flatworm nutrition consists more of a series of intimate relationships between the nature of the food, the structure and function of the pharynx, and the course of digestion, than of a simple increase in complexity from primitive to more elaborate forms. Possibly the "amoeboid" method of feeding in the Acoelan *Convoluta* is primitive to the group (*cf.* Hyman, 1951), and no longer found in other forms. Certainly the alternative method in *Convoluta* of using the body as a whole to envelop the prey is still retained in one form or another by the majority of flatworms, with its effectiveness enhanced by elaboration of the pharynx. The limitation of the simple pharynx, found also in the rhabdocoels *Macrostomum* and *Stenostomum,* is that prey has to be engulfed whole and its size is thereby restricted. In contrast, the bulbous pharynx of the rhabdocoel *Mesostoma* can not only ingest small animals but can be forced into larger animals to suck out their contents, though little triturating effect is possible. The cylindrical plicate pharynx of the triclad *Polycelis* is used in a similar but much more effective manner, the whole contents of the prey being withdrawn and discharged into the gut in a finely divided condition. The comparable pharynx of the polyclad *Cycloporus,* however, is used to deliver largely undisrupted food into the gut, the difference being reflected in the subsequent course of digestion. The ruffled plicate pharynx of the polyclad *Leptoplana,* though developmentally akin to the last, is used rather as an extension of the gut in which preliminary breakdown is enzymatic instead of mechanical. Thus the elaboration of the pharynx has made available to the flatworms an increasing range in the size and variety of their food. Mucus plays a minor part in the feeding of the majority, but does serve to hold the prey and facilitate ingestion. In *Polycelis* alone it is used for the actual capture of prey.

The course of digestion is linked with the feeding mechanism, since this determines the condition of the food on entry to the gut. In *Convoluta* the position is anomalous in that digestion in its gut syncytium might be looked upon either as intracellular or as occurring within temporary lumina. In *Polycelis* the food is very finely divided by the pharynx and the particles taken in by phagocytosis for intracellular digestion. No evidence of intraluminar digestion was found in this species, either of mixed food or of the three food elements fed separately, in agreement with Willier, Hyman and Rifenburgh (1925) and Kelley (1931); the contrary opinion of Arnold (1910) was based on the erroneous supposition that the "sphere cells" of the gut were glandular and discharged into the lumen. Some evidence of intracellular digestion in mesenchyme cells was observed, of food apparently received indirectly from the endoderm cells. In striking contrast is the purely intraluminar digestion of *Cycloporus,* which shows that the flatworms are capable of evolving this more advanced process. In all the other types examined some preliminary breakdown of the food, either by mechanical or enzymatic means, occurred in the pharynx or in the gut lumen, to be followed by phagocytosis and intracellular digestion; this preliminary breakdown should perhaps be interpreted as an adaptation to allow the persistence of the more primitive form of digestion.

Experimental feeding with proteins, carbohydrates and fats given separately showed that fully grown *Polycelis* can not only utilize and store all these food elements but can survive upon any one indefinitely. *Polycelis* also forms normal food

reserves of all three types: specific protein reserves in the "sphere cells" of the gut, fat and glycogen in the gut and mesenchyme cells. Glycogen is the first to be used up and appears the least important. In the others fat forms the main food reserve, supplemented by protein in *Macrostomum,* but they are less prominent than in the triclad, and the capacity to withstand starvation is correspondingly reduced.

I wish to thank Professor E. A. Spaul for suggesting this problem and for assistance, and Dr. T. Kerr for constant guidance and encouragement.

Summary

1. A study has been made of the food, feeding mechanisms, digestion and food storage in the triclad *Polycelis cornuta,* supplemented by observations on representatives of the other three flatworm orders.

2. Each form has a characteristic method of feeding, but in general the range of available prey has been greatly increased by elaborations in the structure and use of the pharynx. Mucus plays a minor part in feeding, except for the "snares" of the triclad. Rhabdoid material has no function in feeding. It forms a temporary cuticle and in polyclads a covering for the eggs.

3. In *Polycelis* mechanical breakdown of the food is followed by phagocytosis into the gut cells and intracellular digestion. Elsewhere the preliminary breakdown may be wholly or in part enzymatic; only in *Cycloporus* is there purely intraluminar digestion with absorption in place of phagocytosis.

4. *Polycelis* can make use of all three food elements, and stores of each type normally occur. In the other forms food storage is less well developed though reserve fat at least is usually to be found.

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